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New records in the *Tubeufiaceae* from Andean Patagonian forests of Argentina

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Abstract — *Tubeufiaceae* (*Pleosporales*, *Ascomycota*) occurring on native trees from the Andean Patagonian forests in Argentina are described and illustrated. *Acanthostigma minutum* and *Tubeufia cerea* with its anamorphic state are reported from South America for the first time on *Nothofagus dombeyi* and *N. antarctica*, respectively. Both species were up to now only known from the Northern Hemisphere.

Key words — ascomycetes, *Helicosporium, Nothofagaceae*

Introduction

Barr (1979) erected the family *Tubeufiaceae* to accommodate several genera of *Pleosporales* that are saprobic on wood, hypersaprobic or hyperparasitic on other fungi, or parasitic on scale insects. The ascomata are small, pallid yellowish to brownish, globose, conic, ellipsoid or cylindrical. She included six genera in the family: the type genus *Tubeufia*, *Letendraea* Sacc., *Melioliphila* Speg., *Podonectria* Petch, *Rebentischia* P. Karst., and *Thaxteriella* Petr. Later, Barr (1980) added five more genera — *Allonecte* Syd., *Boerlagiomyces* Butzin, *Byssocallis* Syd., *Paranectriella* (Henn. ex Sacc. & D. Sacc.) Höhn., and *Puttemansia* Henn. — and synonymized *Thaxteriella* with *Tubeufia*.

The classification of the *Tubeufiaceae*, mainly based on morphology, has been controversial. Eriksson (2005) placed this family as "*Dothideomycetes* et *Chaetothyriomycetes* incertae sedis". Based on sequence analyses Kodsueb et al. (2006) considered the *Tubeufiaceae* a distinct monophyletic family that clusters within the *Pleosporales* as originally proposed by Barr (1980) and excluded *Boerlagiomyces* and *Letendraea* as phylogenetically unrelated. At the same time Tsui & Berbee (2006) arrived at similar results analyzing molecular data of several *Tubeufia* taxa and helicosporous fungi that are considered anamorphic

states of *Acanthostigma* and *Tubeufia*. They indicated that many *Tubeufia* spp. and most species of *Helicoma*, *Helicomyces,* and *Helicosporium* lay within a strongly supported monophyletic lineage, the *Tubeufiaceae* sensu stricto.

Most species of *Tubeufiaceae* are considered tropical, but there are species that occur primarily in temperate areas (Hughes 1978; Rossman 1979, 1987; Samuels et al. 1978). Additional austral records are known in Argentina, Brazil, Chile, and Paraguay, i.e. four *Acanthostigma* species described by Spegazzini (1884, 1887, 1899, 1909), *Rebentischia costi* in Brazil (Batista et al. 1963), and *Rebentischia massalongoi* recently recorded in Argentina (Bianchinotti & Sánchez 2009).

Rebentischia is an accepted member of *Tubeufiaceae* (Kodsueb et al. 2006) but the position of *Acanthostigma* is doubtful. De Notaris established the genus in 1863 with *A. perpusillum* as type. From the beginning its taxonomic status was confused, with the genus referred first to the *Sphaeriaceae* by Saccardo (1883), to the *Trichosphaeriaceae* by Ellis & Everhart (1892), and then synonymized with *Tubeufia* by Arx & Müller (1975). Barr (1980) regarded *Acanthostigma* as a section in *Tubeufia* but later (Barr 1990, 1993) returned it to the *Trichosphaeriaceae* based on its unitunicate asci. Crane et al. (1998) established another genus in the *Tubeufiaceae*, *Acanthostigmina* Höhn. More recently, Réblová & Barr (2000) examined the type material of *Acanthostigma* and confirmed its position in *Tubeufiaceae,* citing *Acanthostigmina* as a synonym. Tsui et al. (2006) found that *A. perpusillum* clusters with *Tubeufia cerea*, and they suggested that *Tubeufia* should be synonymized under *Acanthostigma*.

We have re-examined the specimens representing *Acanthostigma* described by Spegazzini that have not been included in former revisions of the genus. Also, we describe and illustrate other representatives of the *Tubeufiaceae* s.l. collected on native trees from the Andean Patagonian forests. *Acanthostigma* and *Tubeufia* are recorded for the first time in Argentina, with *Acanthostigma minutum* and *Tubeufia cerea* reported for the first time in South America. These new records expand the geographical distribution of the family to the most austral point.

Materials and methods

The samples were collected in forests of Los Alerces National Park (Chubut) and Lanín National Park (Neuquén) located in the southern Andes of Patagonia (Argentina). The vegetation is composed mostly of native *Nothofagus* species together with some species of *Cupressaceae*, *Proteaceae*, ferns and mosses. The climate is temperate to cold with high humidity. Leaves, small branches and bark showing fungal growth when observed with a field magnifying glass were placed in paper bags and transported to the laboratory. The samples were dried at room temperature and deposited at Bahía Blanca Biology Herbarium

(BBB). For microscopic examinations sections were hand-made and mounted in water or 5% KOH with phloxine. At least ten measurements were taken for each structure and all were made in tap water. Calcofluor 1% was used for the examinations made under the fluorescence microscope. The LPS and NYBG Herbaria provided type material. Herbarium abbreviations follow Holmgren et al. (1990). The term "x_{av}" represents the average dimension.

Results and discussion

Acanthostigma De Not., Sfer. Ital., Cent. I, Fasc. 2: 85. 1863.
Type species: *A. perpusillum* De Not.

Acanthostigma minutum (Fuckel) Sacc., Syll. Fung. 2: 209. 1883. figs. 1–7

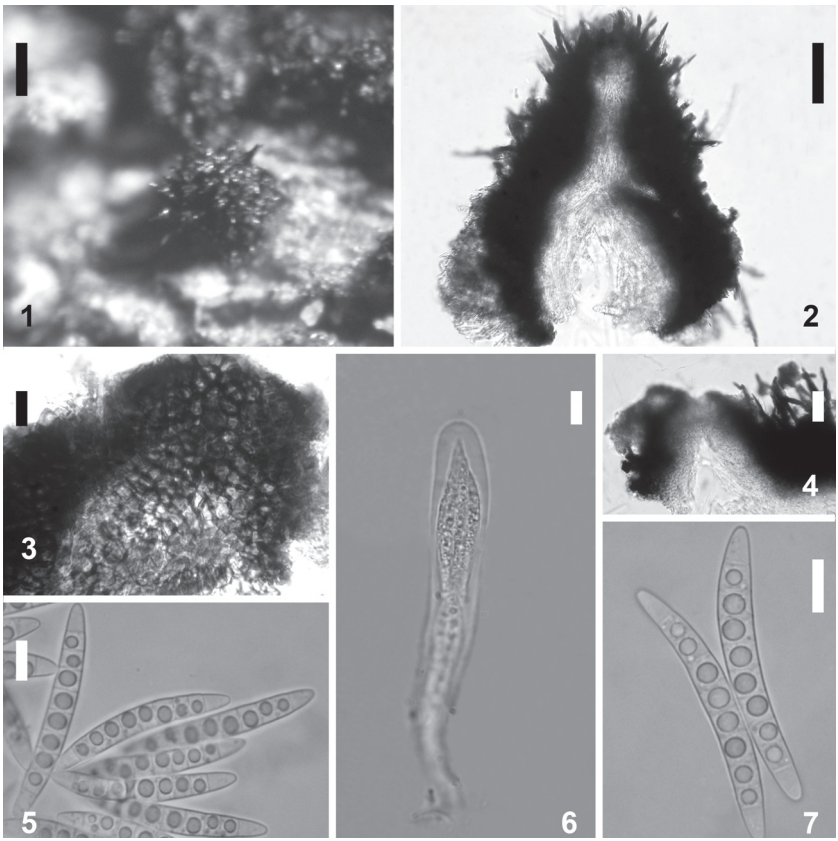
Ascomata superficial to semi-immersed under periderm, globose to subglobose, dark brown to black when dried, (95–)110–300(–350) × 100–300(–330) µm; ostiole papillate, 61–112.5 × 35.7–47.5 µm; surface covered with setae, straight or curved, most densely distributed on upper half, 0–1 septate, dark brown, 46–130 µm long (x_{av} = 79 µm), 3–8.2 µm wide at base (x_{av} = 6 µm), 1–3.8 µm wide at apex (x_{av} = 1.9 µm). peridium of *textura angularis*, two–layered in longitudinal section, outer layer composed of 3–5 rows of thick–walled, dark brown cells, inner layer composed of 2–4 rows of light brown cells, 18–55 µm thick (x_{av} = 35 µm). pseudoparaphyses cellular ramified, anastomosed, septate, hyaline, 1–3 µm thick. periphysoides cellular ramified, septate, hyaline, ca. 2.5 µm thick. asci bitunicate, cylindrical to clavate, 80–150 × 12–50 µm (x_{av} = 116 × 22 µm), 8–spored. ascospores elongate fusiform, tapering to ends and rounded, symmetric, straight or slightly curved, (7–) 9 (–12) septate, not constricted or slightly constricted at septa, hyaline, smooth, 35–68(–75) × 5–8 µm (x_{av} = 50 × 6.8 µm). Anamorph — *Helicomyces* sp. (not seen).

Distribution — America (Argentina, Canada, USA); Asia (China, Taiwan); Europe (France, Germany, Switzerland).

Ecology — on decaying wood of deciduous trees and woody, dicotyledonous shrubs and on old ascomata of other ascomycetes. Recorded on *Fagus sylvatica* L., *Gaultheria shallon* Pursh, *Nothofagus dombeyi* (Mirb.) Oerst., *Populus* sp., and *Quercus* sp.

Material examined — **ARGENTINA**: Neuquén, Parque Nacional Lanín, on the way to Hui Hui lake, on old xylariaceous stromata on bark of *Nothofagus dombeyi*, 17. V. 2007, leg. MV Bianchinotti and RM Sánchez 579 (BBB), Paso del Cordoba, on bark of *N. dombeyi*, 18. I. 2009, leg. MV Bianchinotti and RM Sánchez 776 and 781 (BBB). **USA**: Connecticut, 1 mi south of Canaan, on decayed wood associated with *Hemitrichia clavata*, 2. XI. 1959, CT Rogerson, (as *Acanthostigma decastylum*, NY).

Comments — *Acanthostigma minutum* is recorded for the first time in South America. It was found without its anamorph growing on old xylariaceous



Figures 1–7. *Acanthostigma minutum* (from MVB–RS 579, 776 & 781, deposited in BBB). 1. Ascoma on bark of *Nothofagus dombeyi*. 2. Longitudinal section. 3. Peridium. 4. Section of papilla. 5, 7. Ascospores. 6. Ascus. Bars: 1 = 100 µm. 2 = 50 µm. 4 = 20 µm. 3, 5–7 = 10 µm

stromata on *Nothofagus dombeyi* logs. We compared our specimens with a collection from the USA authenticated by Réblová & Barr (2000), which differs in having ascospores with more septa (10–14). This is the first record of the genus in Argentina.

Excluded and doubtful species

Acanthostigma dimerosporioides Speg., Anal. Mus. Nac. Bs. As. 6: 277. 1898.

figs. 8–9

Ascomata superficial, globose, setose, dark brown, 155–158 µm diam, ostiole circular, 28 µm diam. setae septate, brown, 50–250 × 4–5 µm (fide Spegazzini

1898). ASCI bitunicate. PSEUDOPARAPHYSES not seen. ASCOSPORES fusiform, 3–septate, dark brown, smooth, 18.5–20 × 5 µm.

MATERIAL EXAMINED — **ARGENTINA**, La Plata, on *Gnaphalium purpureum*, III 1899, leg. CL Spegazzini (LPS 2667!).

COMMENTS — The 3–septate, dark brown ascospores exclude this material from *Acanthostigma*. We think the specimen probably belongs to *Herpotrichiellaceae*.

Acanthostigma gnaphaliorum Speg., Anal. Mus. Nac. Bs. As. 19: 375. 1909.

FIGS. 10–11

Ascomata superficial, globose, setose, dark brown, 169–170 µm diam, ostiole circular, 35 µm diam. SETAE 2–8 septate, brown to pale brown, 95–145 µm long and 3.5–5 µm wide at base. ASCI unitunicate, without any visible apical apparatus, 50–60 × 8 µm. PSEUDOPARAPHYSES not seen. ASCOSPORES fusiform, with one inner cell slightly broader, 3–septate, pale brown, smooth.

MATERIAL EXAMINED — **ARGENTINA**, La Plata, Ensenada, on *Gnaphalium purpureum*, 28. XI. 1906, leg. CL Spegazzini (LPS 2391!).

COMMENTS — Only immature ascospores still in the asci were seen. Spegazzini described it as aparaphysate. This species does not belong to the *Tubeufiaceae* because of the combination of unitunicate asci and 3-septate, pale brown ascospores. It resembles members of the *Chaetosphaeriaceae*.

Acanthostigma imperspicuum Speg., Bol. Acad. Nac. Ci. Córdoba 11: 46. 1887.

FIGS. 12–16

ASCOMATA superficial, globose, setose, reddish dark brown, 189–202 µm diam. SETAE dark brown, 38 µm long. ASCI not seen. ASCOSPORES fusiform, with one inner cell slightly broader, 3–septate, pale brown, smooth, 13–16 × 4–5 µm.

MATERIAL EXAMINED — **CHILE**, Patagonia, Cabo Negro, on *Fagus antarcticum* [≡ *Nothofagus antarctica*], VI 1886, leg. CL Spegazzini (LPS 87!).

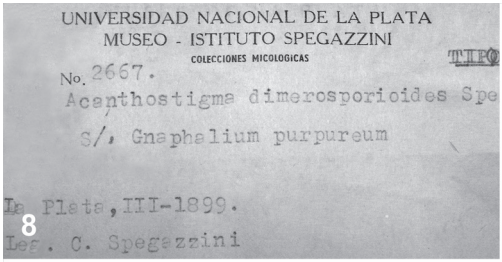
COMMENTS — The material is in poor condition and has no asci; however, Spegazzini illustrated the asci as unitunicate. Because of the combination of characters, it probably belongs in the *Chaetosphaeriaceae*.

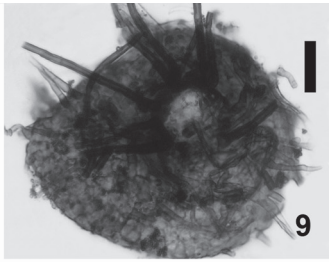
Acanthostigma guaraniticum Speg., Anal. Soc. Ci. Argent. 18(6): 286. 1884.

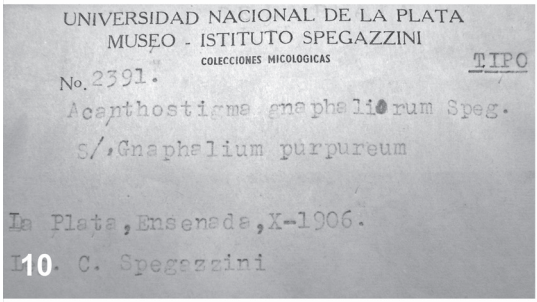
FIGS. 17–20

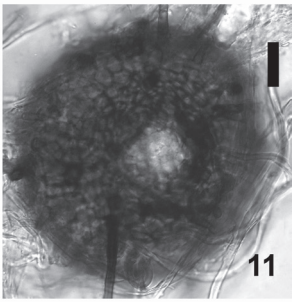
MATERIAL EXAMINED — **PARAGUAY**, Caá–Guazú, on *Eugenia* sp., Leg. Balanza 3452, I 1882, det. CL Spegazzini (LPS 2664!).

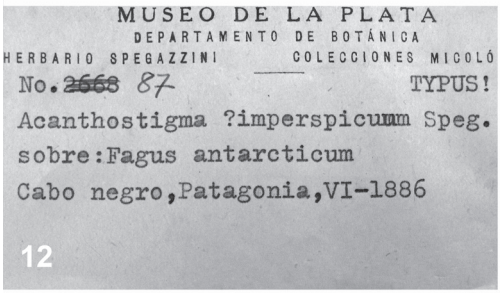
COMMENTS — This material is very scarce and consists of a single, small leaf of *Eugenia* sp. We could not find any ascomata. Brown conidiophores were observed, but no conidia were found. According to Spegazzini´s drawings on the type envelope (available also in Arambarri et al. 2008), this species resembles

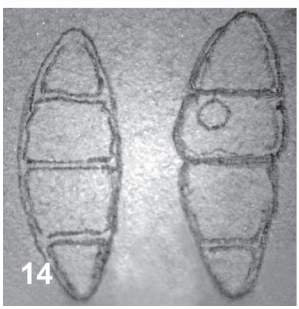


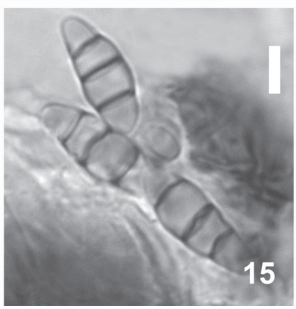


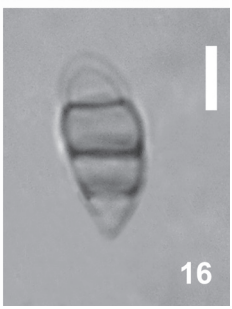




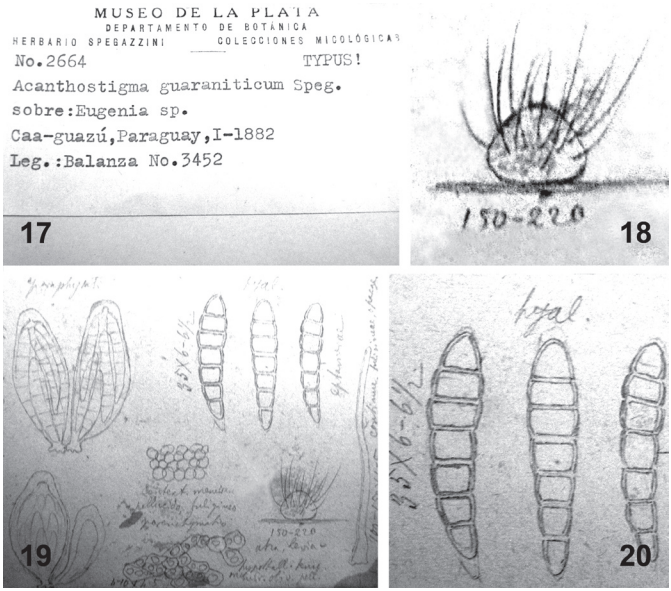








Figures 8–16. Spegazzini's herbarium material (from LPS). 8, 10 and 12. Envelopes of the types. 8–9. *Acanthostigma dimerosporioides* (LPS 2667!). 9. Ascoma top view. 10–11. *A. gnaphaliorum* (LPS 2391!). 11. Ascoma top view. 12–16. *A. imperspicuum* (LPS 87!). 13. Ascoma side view. 14. Drawing of ascospores done by Spegazzini on type envelope. 15–16. Ascospores (LPS 87!).
Bars: 9, 11, 13 = 20 µm. 15–16 = 3 µm.



Figures 17–20. *Acanthostigma guaraniticum* (from LPS 2664!). 17. Envelope of the type. 18–20. Original illustrations from type envelope. 18. Superficial and setose ascoma. 19. Summary of all the structures and tissues. 20. Ascospores.

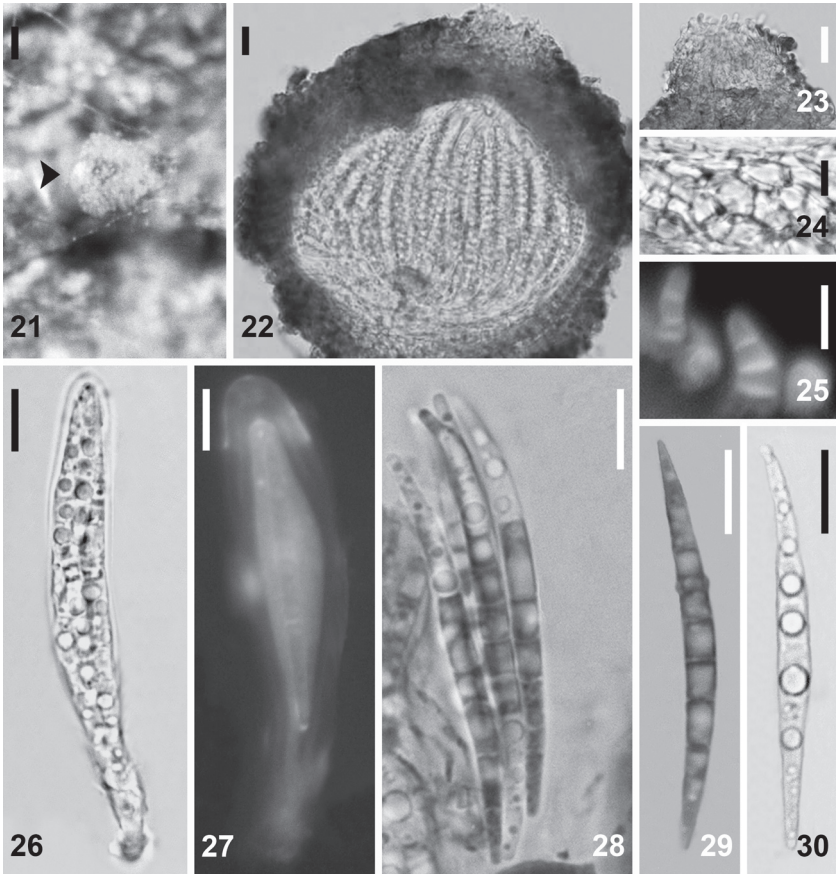
a true *Acanthostigma*. However, Spegazzini described the asci as unitunicate (Spegazzini 1884) so, until authentic material is located, this should be treated as a species dubia.

Tubeufia Penz. & Sacc., Malpighia 11: 517. 1897.

Type species: *T. javanica* Penz. & Sacc. [= *T. paludosa* (P. Crouan & H. Crouan) Rossman].

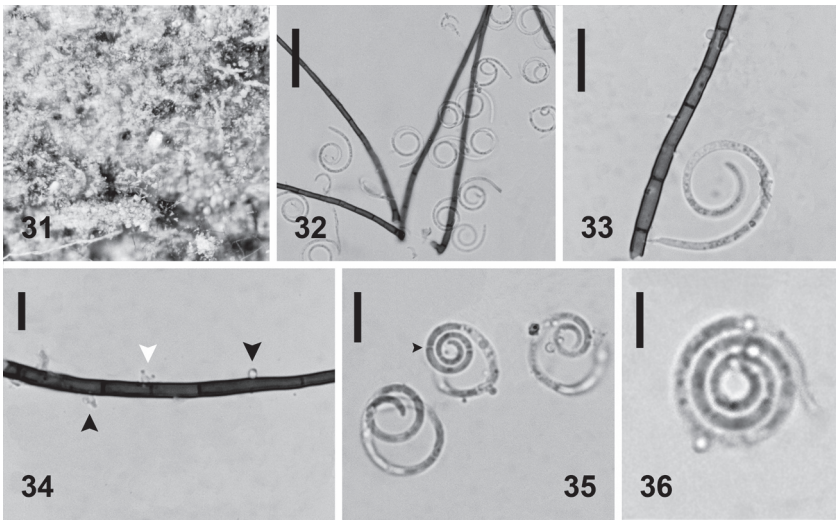
Tubeufia cerea (Berk. & M.A. Curtis) Höhn., Sitzungsber. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 128: 562. 1919. figs. 21–36

Ascomata globose or collabent when dried, bright yellow becoming yellowish brown towards base, a little darker in depression around the papilla, 170–225 × 80–215 µm (x_{av} = 200 × 130 µm); papilla 30–50 × 55–100 µm (x_{av} = 40 × 77.5 µm); ostiole circular, 30–40.8 µm (x_{av} = 36 µm); surface pulverulent with protruding conical cells, sometimes curved, with 1–3 septa, yellow, 5–8 × 4–5 µm. peridium 20–50 µm thick (x_{av} = 36 µm), forming *textura angularis*, cells 5–10 × 3–8 µm (x_{av} =7.6 × 5 µm). asci bitunicate, cylindrical to claviform, 76–87 × 10–13 µm (x_{av} = 81.4 × 11.4 µm). pseudoparaphyses narrow, ramified, parallel or somewhat interwoven, septate, 1–2 µm thick. ascospores elongate fusiform, often curved, apices acute, 6–11 septate, not constricted at septa, hyaline or slightly yellowish, smooth, 38.8–58 × 3–6 µm (x_{av} = 48.7 × 4 µm).



Figures 21–30. *Tubeufia cerea* (from MVB–RS 564, deposited in BBB). 21. Ascoma (arrow head) on bark of *Nothofagus antarctica*. 22. Longitudinal section. 23. Papilla. 24. Peridium. 25. Protruding conical cells of the ascomal wall showing the septa using fluorescence microscopy. 26. Ascus 8–spored (in water). 27. Ascus viewed with fluorescence microscopy. 28–30. Ascospores (28–29 in phloxine, 30 in water). Bars: 21 = 50 µm, 23 = 20 µm, 22, 26–30 = 10 µm, 24–25 = 5 µm.

Anamorph — *Helicosporium virescens* (Pers.) Sivan. Colonies effuse, forming a loose, cottony layer, yellow to greenish yellow. mycelium scarcely ramified, hyaline to pale brown, 2–10 µm wide. conidiophores erect, unbranched, dark brown basally, pale brown to hyaline towards the setiform sterile apex, 61–210 µm long, 2.5–10 µm wide at base, 2–2.5 µm wide at apex. conidiogenous cells integrated, mono or polyblastic, 7–21 × 2–3 µm. conidia cochleated, coiled 2–3 times, multiseptate, hyaline, smooth, coils 10–21 µm diam (x_{av} = 16.6 µm), cells 1.5–2 µm wide.



Figures 31–36. *Tubeufia cerea* anamorph: *Helicosporium virescens* (from MVB–RS 564, deposited in BBB). 31. Colony on *Nothofagus antarctica*. 32. Conidiophores and conidia. 33. Conidium attached to conidiophore. 34. Conidiophore showing two monoblastic conidiogenous denticles (black arrows) and a polyblastic denticle (white arrow). 35. Conidia with septa (arrow points to a septum). 36. Conidia coiled three times. Bars: 32 = 20 µm. 33–35 = 10 µm. 36 = 5 µm.

Distribution — Africa (Congo); Asia (USSR, India); America (Argentina, Canada, Guyana, Puerto Rico, USA); Europe (Austria, Belgium, Finland, France, Germany, Netherlands, Poland, Portugal, Sweden, UK).

Ecology — On wood and bark lying on the ground, on herbaceous substrates, and on old ascomata or mycelium of other ascomycetes.

Material examined — **ARGENTINA**, Neuquén, Parque Nacional Lanín, RN 234 near Villa Traful access route, on log of *Nothofagus antarctica* (G. Forst) Oerst., 16. V. 2007, leg. MV Bianchinotti and RM Sánchez 564 (BBB).

Comments — The genus *Tubeufia* was erected in 1897 by Penzig and Saccardo to accommodate three species from Java (i.e., *T. javanica*, *T. coronata, T. anceps*) characterized by white, cream-pink to brownish, vertically oblong to ovoid ascomata and cylindrical, fusiform to vermiform, multiseptate ascospores.

This is the first record of *Tubeufia* in Argentina and the first time that *Tubeufia cerea* is recorded in South America. Our material differs from that described by Munk (1957) in having larger ascospores (36–48 × 2.5–3.5 µm). *Tubeufia cerea* is widely distributed in temperate areas in the Northern Hemisphere, with a few records from the tropics (Cannon 1999). This is the first report of this species in the subpolar zone.

Other species of *Tubeufia* previously recorded in South America are *T. albo-ostiolata* Rossman in Venezuela, *T. amazonensis* Samuels et al. in Brazil, and *T. aurantiella* (Penz. & Sacc.) Rossman, *T. cylindrothecia* (Seaver) Höhn., *T. helicoma* (W. Phillps & Plowr.) Piroz., *T. palmarum* (Torrend) Samuels et al., and *T. paludosa* in Brazil and Venezuela (Rossman 1987, Samuels et al. 1978).

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